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Drug Uptake in a Rodent Sarcoma Model after Intravenous Injection or Isolated Lung Perfusion of Free/Liposomal Doxorubicin

THESE

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Drug Uptake in a Rodent Sarcoma Model after Intravenous Injection or Isolated Lung Perfusion of Free/Liposomal Doxorubicin

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Rapport de synthèse

Drug uptake in a rodent sarcoma model after intravenous injection or isolated lung perfusion of free/liposomal doxorubicin

Introduction

La distribution de doxorubicine libre et doxorubicine liposomale pegylée (Liporubicin^{TM}) a été comparée après administration intraveineuse ou application via perfusion isolée du poumon (ILP) dans le parenchyme pulmonaire et dans la tumeur des poumons de rongeurs, porteurs d'une tumeur sarcomateuse.

Matériel et méthode

Une tumeur sarcomateuse unique a été générée dans le poumon gauche de 36 rongeurs (Fisher rats) suivie, 10 jours plus tard, par application de doxorubicine ou Liporubicin^{TM} soit par perfusion isolée du poumon (n = 20) ou administration intraveineuse (n = 12). Deux différentes concentrations ont été utilisées (100 µg et 400 µg) à doses équimolaires pour les deux formulations de doxorubicine. La concentration des agents cytostatiques ont été mesurées dans la tumeur et le parenchyme pulmonaire à l'aide de chromatographie (HPLC).

Résultats

Les résultats indiquent que pour doxorubicine libre, le taux de concentration dans la tumeur et le parenchyme pulmonaire est 3 fois (dosage de 100 µg) et 10 fois (dosage de 400 µg) plus élevé après ILP par rapport à l'administration intraveineuse. En revanche, pour Liporubicin^{TM}, le taux de concentration est similaire dans la tumeur et le parenchyme pulmonaire entre ILP et administration intraveineuse, pour les deux doses appliquées.

Conclusion

Pour ILP et administration intraveineuse, le ratio entre accumulation de l'agent cytostatique dans la tumeur versus dans le parenchyme pulmonaire a été comparé pour les deux formulations de doxorubicine ainsi que pour les deux dosages. Pour les deux formulations et dosages de doxorubicine, ILP aboutit à un ratio plus élevé par rapport à l'administration intraveineuse. Cependant, pour les deux formulations et dosages de doxorubicine, ILP résulte également en une distribution de l'agent cytostatique plus hétérogène dans le parenchyme pulmonaire comparé à l'administration intraveineuse.

En résumé, l'application de doxorubicine par ILP aboutit donc à une accumulation tumorale élevée et à une augmentation du ratio tumeur-parenchyme pulmonaire, mais en même temps également à une distribution plus hétérogène dans le parenchyme pulmonaire par rapport à l'application intraveineuse. Ceci a été observé pour les deux formulations de doxorubicine et pour les deux dosages appliqué.

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Institutional report - Experimental

Drug uptake in a rodent sarcoma model after intravenous injection or isolated lung perfusion of free/liposomal doxorubicin☆,☆☆

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Abstract

The distribution of free and liposomal doxorubicin (Liporubicin™) administered by intravenous injection (IV) or isolated lung perfusion (ILP) was compared in normal and tumor tissues of sarcoma bearing rodent lungs. A single sarcomatous tumor was generated in the left lung of 35 Fischer rats, followed 10 days later by left-sided ILP ($n=20$) or IV drug administration ($n=12$), using 100 μg and 400 μg free or liposomal doxorubicin, respectively. The tumor and lung tissue drug concentration was measured by HPLC. Free doxorubicin administered by ILP resulted in a three-fold (100 μg) and 10-fold (400 μg) increase of the drug concentration in the tumor and normal lung tissue compared to IV administration. In contrast, ILP with Liporubicin™ resulted in a similar drug uptake in the tumor and lung tissue compared to IV injection. For both drug formulations and dosages, ILP resulted in a higher tumor to lung tissue drug ratio but also in a higher spatial heterogeneity of drug distribution within the lung compared to IV administration. ILP resulted in a higher tumor to lung tissue drug ratio and in a more heterogeneous drug distribution within the lung compared to IV drug administration.

Keywords: Isolated lung perfusion; Intravenous drug application; Sarcoma; Chemotherapy; Doxorubicin; Liposomal encapsulation

1. Introduction

Isolated lung perfusion (ILP) has been recognized as an attractive treatment concept against lung metastases since it specifically delivers a cytostatic agent to the target organ while sparing the systemic circulation. Doxorubicin-based ILP was shown to cause a high drug uptake in the lungs with minimal leakage in the systemic circulation and toxicity [1–5]. However, we previously observed that the penetration of doxorubicin or Liporubicin™ – a pegylated liposomal encapsulated formulation of doxorubicin – administered by ILP was significantly lower in sarcomas grown in rodent lungs compared to normal lung parenchyma [6]. We have also recently shown that doxorubicin-based ILP can lead to a heterogeneous drug distribution within the perfused lung and a high variability in drug distribution between animals [7, 8]. This could, in part, explain the inconsistent results obtained in patients undergoing ILP for lung metastases, irrespective of the cytostatic agent and drug dose used.

Here, we hypothesized that the concentration, formulation and administration of doxorubicin affects its distribution in normal and tumor tissues and could help optimize the tumor to lung tissue drug ratio. Using our previously described sarcoma model, we compared the tumor/normal parenchyma drug uptake following IV or ILP administration of doxorubicin and Liporubicin™. For each condition, we determined the drug uptake in tumors and lung parenchyma by HPLC, the tumor/lung ratio and the drug distribution variability in different lung compartments.

2. Material and methods

Male Fischer rats (Charles River, France) weighing 250–300 g were used. They were treated in accordance with the Animal Welfare Act, the National Institute of Health 'Guidelines for the Care and Use of Laboratory Animals' and according to the Local Ethical Committee of the University of Lausanne.

Thirty-five Fischer rats underwent tumor implantation in their left lung using a syngeneic methylcholanthrene-induced sarcoma (MCA) cell line [9] followed 10 days later by isolated lung perfusion (ILP, $n=20$) or intravenous administration (IV, $n=12$) of free or liposomal encapsulated

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doxorubicin (Liporubicin™) as previously described [6, 9]. ILP and IV drug administration were performed for both drug formulations at equimolar drug doses of 100 μg and 400 μg, respectively. Sixty minutes following ILP and 70 min following IV injection, the animals were sacrificed and the lungs were harvested. Drug concentrations in the tumor and the normal surrounding lung were assessed by high performance liquid chromatography (HPLC) as previously described [6, 7, 10, 11]. In addition, the concentrations of doxorubicin in tissues were separately determined in the upper, middle and lower part of each perfused lung to assess the coefficient of variation (CV%) as previously described [6]. Three untreated animals served as controls for histological and immunohistochemical analysis.

Doxorubicin concentrations in tumors and normal lung parenchyma were compared for both doxorubicin formulations using a Student's *t*-test for unrelated samples. Variabilities in doxorubicin lung tissue levels were expressed as the coefficients of variation of tissue concentration in the three parts of perfused lungs for both doxorubicin formulations and drug doses and were compared using the Student's *t*-test. A bidirectional hypothesis was applied and significance accepted at $P<0.05$.

3. Results

3.1. Controls

Histological assessment of untreated tumors revealed a well-circumscribed sarcomatous tumor in each lung, mainly formed by large undifferentiated cells. Immunostaining for von Willebrand factor revealed an extensive vascular network composed of small vessels and capillaries and present throughout the tumors as previously described [6]. Spontaneous necrosis was observed in <2% of the tumor volume for each case analyzed. Tumor growth within the pleural cavity was not identified in any of the animals.

3.2. Drug concentrations in tumor and lung tissue (*Tables 1 and 2*)

Free doxorubicin administered by ILP resulted in a three- (100 μg) to 10-fold (400 μmg) increase of drug concentration in the tumor ($P=0.048$) and in the lung ($P=0.0002$) compared to IV drug administration. However, doxorubicin concentrations in tumors were always lower than in lung tissue in all animals, for both drug doses and modes of drug administration assessed.

In contrast, Liporubicin™ resulted for each drug dose in similar drug concentrations in the tumor and normal lung tissues following ILP or IV administration. Interestingly, the increase of Liporubicin™ drug dose did not affect tumor or normal tissue drug concentration following ILP but significantly increased these parameters following IV application. Liporubicin™ concentrations in tumors were always lower than in the normal lung tissue, for both drug doses and both modes of drug administration assessed.

Table 1
Tissue concentration, ratio of tumor to normal tissue drug concentration and coefficient of variation (CV%) of free and liposomal encapsulated doxorubicin after isolated lung perfusion (mean values±S.D.)

	Doxorubicin		Liporubicin™	
	100 μg	400 μg	100 μg	400 μg
Tissue concentration (μg/g)				
Lung	13.8±4.3	58.5±20.1	2.0±0.7	5.2±3.7
Tumor	3.9±2.5	36.9±10.4	0.8±0.5	3.2±3.5
Ratio	0.27±0.1	0.67±0.2	0.53±0.5	0.54±0.2
CV%*	27%	24%	49%	28%

*CV%, coefficient of variation of doxorubicin levels measured in the upper, middle, and lower tumor-free part of the perfused lung.

Table 2
Tissue concentration, ratio of tumor to normal tissue drug concentration and coefficient of variation (CV%) of free and liposomal encapsulated doxorubicin after IV drug application (mean values±S.D.)

	Doxorubicin		Liporubicin™	
	100 μg	400 μg	100 μg	400 μg
Tissue concentration (μg/g)				
Lung	3.7±0.2	10.8±0.8	1.8±0.6	8.2±0.2
Tumor	1.5±0.2	2.9±0.8	0.5±0.02	3.4±0.2
Ratio	0.39±0.04	0.27±0.1	0.28±0.1	0.41±0.04
CV%*	13%	5%	12%	11%

*CV%, coefficient of variation of doxorubicin levels measured in the upper, middle, and lower tumor-free part of the perfused lung.

3.3. Ratio of tumor to lung tissue drug concentration (*Fig. 1*)

To determine how the doxorubicin administration, formulation or dose affected its penetration in tumors, we determined the tumor to normal tissue drug ratio for each of the previous experiments. *Free doxorubicin:* increasing the drug dose during ILP significantly improved the tumor to lung tissue drug ratio ($P=0.003$). This holds not true for IV application of free doxorubicin. Comparing IV application with ILP, the ratio was significantly higher for ILP at a drug dose of 400 μg ($P=0.02$) but not at a dose of 100 μg.

Liporubicin™: ILP revealed for both drug doses a higher tumor to lung tissue drug ratio than IV application although the differences were not significant. After ILP, both drug doses resulted in a similar ratio; after IV administration, the ratio was higher with the higher drug dose but not statistically significant ($P=0.06$).

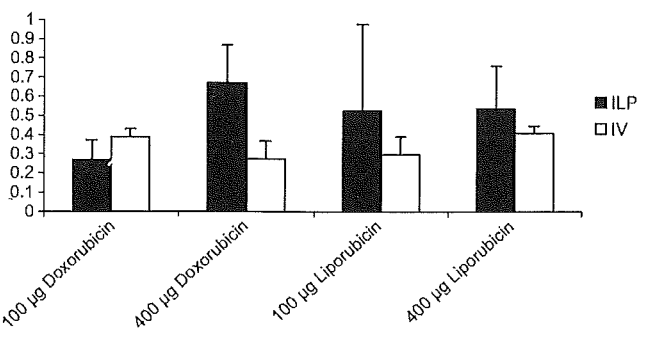

Fig. 1. Ratio of tumor to lung tissue drug concentration in sarcoma bearing lungs for ILP and IV drug administration of 100 μg and 400 μg doxorubicin and equimolar-dosed Liporubicin™.

3.4. Spatial drug distribution after ILP and IV drug administration

ILP led to a wide inter-animal variability and a heterogeneous spatial drug distribution within the perfused lung for both doxorubicin formulations at both drug doses as demonstrated by the large coefficients of variation (CV%) (Table 1). Both, inter-animal variability and spatial drug distribution was less pronounced after IV drug application, for both drug formulations (Table 2).

4. Discussion

Since advanced sarcoma often presents with lung metastases without extra thoracic tumor manifestation, doxorubicin-based ILP has been assessed under clinical and experimental conditions as an alternative method for the delivery of high-dose chemotherapy while minimizing systemic toxicity. Although effective drug delivery to the perfused lung was obtained, ILP has failed to demonstrate effective tumor control in clinical trials [1–3]. We have previously shown that ILP with doxorubicin results in an uneven distribution of drug within the perfused lung [6–8] and that little doxorubicin penetrates tumors in a rodent model of sarcoma metastasis to the lung [6, 7]. Fluorescence microscopic assessment revealed that doxorubicin fails to cross the endothelial barrier of vessels within the tumors during ILP in this model [7].

Here, we compared the drug uptake in tumors and normal lung parenchyma, the tumor to normal lung tissue drug ratio and the pattern of drug distribution in the lung after ILP and IV drug administration of doxorubicin and equimolar dosed Liporubicin™ in a rat model of sarcoma metastasis to the lungs. Histological assessment and immunostaining for von Willebrand's factor showed that untreated sarcomatous tumors were well-circumscribed, had a rich vascular network consisting of fine branching capillaries witnessing the presence of a well developed tumor vascularization and did not present spontaneous necrosis.

Doxorubicin administered by ILP resulted in a three- (100 μg) to 10-fold (400 μg) increase in drug concentration in the lung and tumor tissues compared to IV administration. However, both modes of doxorubicin administration resulted in a consistently lower drug uptake in tumors than in normal lung tissue, for both drug doses applied. The highest tumor to lung tissue drug ratio was obtained with 400 μg of doxorubicin administered by ILP, however, the spatial distribution of doxorubicin in the lung parenchyma after ILP was highly variable within animals and between animals as previously reported in rodent [7] and porcine [8] models.

We then conducted the same experiments using a pegylated liposomal encapsulated form of doxorubicin (Liporubicin™). Liposomal delivery systems for anthracyclines have shown several advantages for intravenous application compared with the administration of the free drug. They have been shown to improve drug delivery to tumors while decreasing toxicity to normal tissues [12–14]. The tumor-targeting mechanism relies on the size of liposomes, which render them difficult to extravasate through the capillaries of normal tissues outside of the reticulo-endothelial system.

In our study, we found that Liporubicin™-based ILP resulted for both drug doses in a similar tumor and lung tissue drug uptake compared to IV application which may be explained by the specific pharmacokinetic profile of liposomal doxorubicin [12–14]. However, ILP with Liporubicin™ revealed a consistently lower drug uptake in tumors and lung tissue compared to equimolar-dosed doxorubicin. This could be due to the microvascular properties of the sarcoma model used in this study. Indeed, previous work in various tumor types has shown that the convective forces involved in drug distribution can be limited by the high intratumoral interstitial fluid pressure. In this situation, the major driving force becomes diffusion which is mostly affected by the drug molar mass. Here, while the size of Liporubicin™ may increase its specificity for the tumor vasculature compared to doxorubicin, it may also limit its distribution because of the increase in size and molar mass. Finally, another element that could limit the distribution of Liporubicin™ administered by ILP is the absence of enzymes that have the capacity to de-pegylate Liporubicin™ and liberate doxorubicin in the lung perfusion solute.

Conversely, Liporubicin™-based ILP revealed for both drug doses a higher tumor to lung tissue drug ratio than IV application although the differences were not significant due to the great inter-animal variability after ILP. In addition, the tumor to lung tissue drug ratio was not significantly different between doxorubicin and Liporubicin™-based ILP. These findings suggest that Liporubicin™ may have advantages for drug escalating ILP schedules since an increase of the drug dose may allow an enhanced tumor drug uptake without exposing the lung parenchyma to excessive doxorubicin concentrations and to a risk of doxorubicin-induced lung toxicity.

Finally, similarly to doxorubicin, Liporubicin™ administered by ILP was associated with a higher heterogeneity of doxorubicin distribution and inter-animal variability compared to IV application implicating that this phenomenon is related to the mode of drug delivery and cannot be alleviated by liposomal encapsulation of doxorubicin.

In conclusion, ILP administration of both doxorubicin formulations resulted in a trend for better tumor to lung tissue drug ratio than IV administration with a significant increase in this ratio for free doxorubicin at a dose of 400 μg. Although Liporubicin™ administered by ILP did not cause a higher tumor to lung tissue drug ratio compared to IV, its lung concentrations were significantly lower than that of doxorubicin. The latter could thus bear an advantage for investigational ILP protocols with drug-escalating schedules.

References

[1] Minchin RF, Johnston MR, Aiken MA, Boyd MR. Pharmacokinetics of doxorubicin in isolated lung of dogs and humans perfused in vivo. J Pharmacol Exp Ther 1984;229:193–198.

[2] Johnston MR, Minchen RF, Dawson CA. Lung perfusion with chemotherapy in patients with unresectable metastatic sarcoma to the lung or diffuse bronchioloalveolar carcinoma. J Thorac Cardiovasc Surg 1995; 110:368–373.

[3] Burt ME, Liu D, Abolhoda A, Ross HM, Kaneda Y, Jara E, Casper ES, Ginsberg RJ, Brennan MF. Isolated lung perfusion for patients with

unresectable metastases from sarcoma: a phase I trial. Ann Thorac Surg 2000;69:1542–1549.

[4] Weksler B, Ng B, Lenert JT, Burt ME. Isolated single lung perfusion with doxorubicin is pharmacokinetically superior to intravenous injection. Ann Thorac Surg 1993;56:209–214.

[5] Furrer M, Lardinois D, Thormann W, Altermatt HJ, Betticher D, Cerny T, Fikrle A, Mettler D, Althaus U, Burt ME, Ris HB. Isolated lung perfusion: single-pass system versus recirculating blood perfusion in pigs. Ann Thorac Surg 1998;65:1420–1425.

[6] Yan H, Cheng C, Haouala A, Krueger T, Ballini JP, Peters S, Decosterd LA, Letovanec I, Ris HB, Andrejevic-Blant S. Distribution of free and liposomal doxorubicin after isolated lung perfusion in a sarcoma model. Ann Thorac Surg 2008;1225–1232.

[7] Krueger T, Kuemmerle A, Andrejevic-Blant S, Yan H, Pan Y, Ballini JP, Klepetko W, Decosterd LA, Stupp R, Ris HB. Antegrade versus retrograde isolated lung perfusion: doxorubicin uptake and distribution in a sarcoma model. Ann Thorac Surg 2006;82:2024–2030.

[8] Krueger T, Kuemmerle A, Kosinski M, Denys A, Magnusson L, Stupp R, Delaloye AB, Klepetko W, Decosterd LA, Ris HB, Dusmet M. Cytostatic lung perfusion results in heterogeneous spatial regional blood flow and drug distribution. Evaluation of different cytostatic lung perfusion techniques in a porcine model. J Thorac Cardiovasc Surg 2006;132:304–311.

[9] Pan Y, Krueger T, Tran N, Yan H, Ris HB, McKee TA. Evaluation of tumor vascularisation in two rat sarcoma models for studying isolated lung perfusion. Injection route determines the origin of tumor vessels. Eur Surg Res 2005;37:92–99.

[10] Kuemmerle A, Krueger T, Dusmet M, Vallet C, Pan Y, Ris HB, Decosterd LA. A validated assay for measuring doxorubicin in biological fluids in an isolated lung perfusion model: matrix effect and heparin interference strongly influence doxorubicin measurements. J Pharm Biomed Anal 2003;33:475–494.

[11] Chin DL, Lum BL, Sikic BI. Rapid determination of pegylated liposomal doxorubicin and its major metabolite in human plasma by ultraviolet visible high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2002;779:259–269.

[12] Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Matthay K, Huang SK, Lee KD, Woodle MC, Lasic DD, Redemann C, Martin FJ. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc Natl Acad Sci USA 1991;88:11460–11464.

[13] Gabizon AA. Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. Cancer Res 1992;52:891–896.

[14] Lyass O, Uziely B, Ben-Yosef R, Tzemach D, Heshing N, Lotem M, Brufman G, Gabiyon A. Correlation of toxicity with pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in metastatic breast carcinoma. Cancer 2000;89:1037–1047.